CLAIMS

What is claimed is:

1. A cartridge for determining the presence or amount of a microbial contaminant in a sample, the cartridge comprising:

(i) a housing defining a fluid inlet port, an optical cell, and a conduit having a fluid contacting surface for providing fluid flow communication between the fluid inlet port and the optical cell; and

(ii) hemocyte lysate disposed on a region of the fluid contacting surface of the conduit, so that when a sample is applied to the fluid inlet port, the sample traverses the region and solubilizes the hemocyte lysate during transport to the optical cell.

2. The cartridge of claim 1, further comprising a chromogenic substrate disposed on a second region of the fluid contacting surface.

3. The cartridge of claim 2, wherein the second region is downstream of the first region.

4. The cartridge of claim 1, further comprising a preselected amount of an agent representative of the microbial contaminant disposed on the fluid contacting surface of the conduit.

5. The cartridge of claim 4, wherein the agent is disposed on the first region.

6. The cartridge of claim 4, wherein the agent is a bacterial endotoxin or a $(1\rightarrow3)$-β-D glucan.

7. A cartridge for determining the presence or amount of a microbial contaminant in a sample, the cartridge comprising:

(i) a housing defining

a first fluid inlet port, a first optical cell, and a first conduit having a fluid contacting surface for providing fluid flow communication between the first fluid inlet port and the first optical cell, and

a second fluid inlet port, a second optical cell, and a second conduit having a fluid contacting surface for providing fluid flow communication between the second fluid inlet port and the second optical cell;

(ii) a first hemocyte lysate disposed on a first region of the fluid contacting surface of the first conduit, so that when a sample is applied to the first fluid inlet port, the sample traverses the region and solubilizes the first hemocyte lysate during transport to the first optical cell; and

(iii) a second hemocyte lysate disposed on a first region of the fluid contacting surface of the second conduit, so that when sample is applied to the second fluid inlet port, the sample traverses the region and solubilizes the second hemocyte lysate during transport to the second optical cell.

8. The cartridge of claim 7, further comprising a chromogenic substrate disposed on a second region of the fluid contacting surface of the first conduit.

9. The cartridge of claim 8, wherein the second region is downstream of the first region.

10. The cartridge of claim 8, further comprising a chromogenic substrate disposed on a second region of the fluid contacting surface of the second conduit.

11. The cartridge of claim 10, wherein the second region is downstream of the first region.

12. The cartridge of claim 7, further comprising a preselected amount of an agent representative of a microbial contaminant disposed on the fluid contacting surface of the first conduit.

13. The cartridge of claim 12, wherein the agent is disposed on the first region.

14. The cartridge of claim 12, wherein the agent is a bacterial endotoxin or a (1→3)-β-D glucan.

15. A method of detecting the presence of a microbial contaminant in a sample, the method comprising the steps of:

(a) introducing a sample into the sample inlet port of the cartridge of any one of claims 1-6;

(b) permitting the sample to move to the optical cell; and

(c) measuring an optical property of the sample in the optical cell, wherein a change in the optical property is indicative of the presence of a microbial contaminant in the sample.

16. The method of claim 15, wherein the change in optical property is an increase in absorbance of light of a preselected wavelength.

17. The method of claim 15, wherein the change in optical property is a decrease in transmission of light of a preselected wavelength.

18. A method of determining the amount of a microbial contaminant in a sample, the method comprising the steps of:

(a) introducing a sample into the sample inlet port of the cartridge of any one of claims 1-6;

(b) permitting the sample to move to the optical cell;

(c) measuring the time in which a preselected change occurs in an optical property of the sample in the optical cell; and

(d) comparing the time measuring in step (c) against a predetermined standard curve to determine the amount of the microbial contaminant in the sample.

19. The method of claim 18, wherein the change in optical property is an increase in absorbance of light of a preselected wavelength.

20. The method of claim 18, wherein the change in optical property is a decrease in transmission of light of a preselected wavelength.

21. A method of determining the presence of a microbial contaminant in a sample, the method comprising the steps of:

(a) contacting a sample with a hemocyte lysate comprising an activatable enzyme to produce a sample-lysate mixture, whereupon the enzyme becomes activated if the microbial contaminant is present in the sample;

(b) after step (a), contacting the sample-lysate mixture with a substrate for the enzyme to produce a sample-lysate-substrate mixture, such that, if the mixture contains activated enzyme, the activated enzyme produces a change in the substrate;

(c) determining the time in which a preselected change occurs in an optical property of the sample-lysate-substrate mixture, wherein the change in optical property results from a change in the substrate; and

(d) comparing the time determined in step (c) against a predetermined standard curve to determine whether the contaminant is present in the sample.

22. The method of claim 21, wherein the microbial contaminant is selected from the group consisting of a lipopolysaccharide, a bacterial endotoxin, and a glucan.

23. The method of claim 22, wherein the microbial contaminant is a lipopolysaccharide.

24. The method of claim 22, wherein the microbial contaminant is a bacterial endotoxin.

25. The method of claim 22, wherein the microbial contaminant is a glucan.

26. The method of claim 21, wherein the change in optical property is an increase in absorbance of light of a preselected wavelength.

27. The method of claim 21, wherein the change in optical property is a decrease in transmission of light of a preselected wavelength.

28. The method of claim 21, wherein the hemocyte lysate is an amebocyte lysate.

29. The method of claim 21, wherein the hemocyte lysate is a *Limulus* amebocyte lysate.

30. The method of claim 21, wherein the hemocyte lysate is an endotoxin-specific amebocyte lysate.

31. The method of claim 21, wherein the hemocyte lysate is an glucan-specific amebocyte lysate.

32. The method of claim 21, wherein the activatable enzyme is pro-clotting enzyme.

33. The method of claim 21, wherein the activatable enzyme is clotting enzyme.

34. The method in claim 21, wherein the substrate is a chromogenic substrate.

35. The method of claim 34, wherein the chromogenic substrate comprises a para-nitroaniline chromophore.

36. The method of claim 34, wherein the chromogenic substrate comprises Ile-Glu-Ala-Arg-pNA, wherein pNA is a para-nitroaniline group.

37. The method of claim 21, comprising the additional step of measuring the amount of the contaminant in the sample.

38. The method of claim 21, wherein the presence of the microbial contaminant is indicative of a microbial infection or microbial contamination.

39. The method of claim 37, wherein the amount of the contaminant is indicative of a microbial infection or microbial contamination.

40. The method of claim 38 or 39, wherein the microbial infection is a bacterial, yeast, mold, or fungal infection.

41. The method of claim 21, wherein steps (a) and (b) provide a predetermined assay sensitivity and duration.

42. The method of claim 21, wherein steps (a) and (b) are performed in a well defined by a solid support.

43. The method of claim 21, wherein steps (a) and (b) are performed in a cartridge.

44. A method of drying a hemocyte lysate onto a solid support, the method comprising the steps:

(a) applying a volume of a mixture comprising a hemocyte lysate, a resolubilizing agent and an anti-flaking agent to the surface of a solid support; and

(b) drying the mixture onto the solid support.

45. The method of claim 44, wherein the drying step is performed in an environment having a temperature from about 4°C to about 40°C and a relative humidity from about 0% to about 30%.

46. The method of claim 45, wherein the temperature is about 25°C.

47. The method of claim 45 or 46, wherein the humidity is about 5%.

48. The method of claim 44, wherein the resolubilizing agent is a sugar or a salt.

49. The method of claim 48, wherein the sugar is selected from the group consisting of mannitol, mannose, sorbitol, trehalose, maltose, dextrose, and sucrose.

50. The method of claim 44, wherein the anti-flaking agent is a polymer or protein.

51. The method of claim 50, wherein the polymer is selected from the group consisting of polyethylene glycol, polyvinyl pyrolidone, polyvinyl alcohol, serum albumin, and dextran.

52. The method of claim 44, wherein the solid support is a fluid contacting surface of a conduit.

53. The method of claim 44, wherein the mixture further comprises a chromogenic substrate.

54. The method of claim 44 or 53, wherein the mixture further comprises an anti-frothing agent.

55. The method of claim 54, wherein the anti-frothing agent is polyvinyl alcohol or polypropylene glycol.

56. The method of claim 44 or 53, wherein the mixture further comprises a bacterial endotoxin or a $(1\rightarrow3)$-β-D glucan.

57. The method of claim 44, wherein the hemocyte lysate is an amebocyte lysate.

58. The method of claim 57, wherein the amebocyte lysate is a *Limulus* amebocyte lysate.

59. A method of preparing an amebocyte lysate depleted of Factor C activity, the method comprising:

(a) providing a preparation of amebocytes; and

(b) lysing the amebocytes in the presence of at least 0.15 M salt to provide an amebocyte lysate preparation depleted of Factor C activity.

60. The method of claim 59, comprising the additional step of after step (b) removing cellular debris from the amebocyte lysate preparation.

61. The method of claim 59, wherein the salt is comprises a monovalent cation.

62. The method of claim 61, wherein the salt is a sodium or potassium salt.

63. The method of claim 62, wherein the salt is sodium chloride or potassium chloride.

64. The method of claim 59, wherein in step (b) the amebocytes are lysed in the presence of salt at a concentration in the range from about 0.15 M to about 6 M.

65. The method of claim 64, wherein the salt is at a concentration from about 0.25M to about 4 M.

66. The method of claim 65, wherein the salt is at a concentration from about 1 to about 2 M.

67. The method of claim 59, wherein the lysate is substantially free of Factor C activity.

68. The method of claim 59, wherein the lysate retains Factor G activity.

69. The method of claim 68, wherein the lysate is a glucan-specific lysate.

70. An amebocyte lysate substantially free of Factor C activity, wherein the lysate comprises at least about 0.25 M salt, and wherein the lysate is capable of reacting with glucan to produce a coagulin gel.

71. The lysate of claim 70, wherein the salt comprises a monovalent cation.

72. The lysate of claim 71, wherein the salt comprises a sodium ion, a potassium ion, or a combination of sodium and potassium ions.

73. The lysate of claim 70, wherein the lysate is substantially free of Factor C activity.

74. The lysate of claim 70, wherein the lysate comprises from at least about 0.25 M to about 6 M salt.

75. The lysate of claim 74, wherein the lysate comprises from about 0.5 M to about 4 M salt.

76. The lysate of claim 75, wherein the lysate comprises from about 1M to about 2 M salt.

77. A manufacture comprising a solid support having dried thereon a composition comprising a hemocyte lysate and (i) an anti-flaking agent, (ii) an anti-frothing agent, (iii) a resolubilizing agent, (iv) an anti-flaking agent and an anti-frothing agent, (v) an anti-flaking agent and a resolubilizing agent, (vi) an anti-frothing agent and a resolubilizing agent, or (vii) an anti-flaking agent, an anti-frothing agent and a resolubilizing agent.

78. The manufacture of claim 77, wherein said composition further comprises a substrate.